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Percutaneous absorption of aromatic amines and the risk assessment resulting from the dermal pathway

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1. ABSTRACT

Aromatic amines (AA) are compounds of different carcinogenic potency causing occupational bladder cancer. The percutaneous absorption of AA is mostly appraised to be high. Many AA are, therefore, assigned with skin notations. However, for the assessment of the dermal exposure route only little data are available. Additionally, in many studies the skin penetration data for AA are provided as absorbed percentage of applied dose or permeability coefficients, which are less useful in risk assessment. In this overview, the toxicological relevance of percutaneous absorption of AA was evaluated and a percutaneous penetration ranking for some AA is proposed. A continuous skin exposure of hands to AA for a few minutes can exceed the inhalative exposure over 8 hours at occupational threshold limit values in the workplace air. The health risk resulting from the percutaneous absorption of AA can be considerable. Also the dermal exposure to azo dyes, which can be metabolized to AA, should be considered with caution.

2. INTRODUCTION

Aromatic amines (AA) are used in the production of dyes, pharmaceuticals, pesticides, and rubber. They also occur as intermediates. AA are of special interest in occupational medical health surveillance, due to their carcinogenic effects (1). The percutaneous absorption of some chemicals, e.g. the glycol ether 2-butoxyethanol or the lactam N-methyl-2-pyrrolidone, may exceed the uptake by inhalation (2, 3). For further glycol ethers and some alcohols, high percutaneous penetration rates (fluxes) have also been determined (4), indicating that the dermal pathway could be equal or even more important as the uptake by inhalation. The occupational uptake of AA predominantly results from dermal exposure and inhalation. The main exposure route differs among AA and relates especially to physicochemical properties of the compounds (5). In databases for occupational hygienists (6-8), the percutaneous absorption of AA is often appraised as generally good. It was shown that the percutaneous absorption of some AA can lead to a significant

contribution to the overall uptake (9, 10). However, only little data for AA is available.

The exposure to AA in workplaces is mostly controlled by ambient air monitoring. The percutaneously absorbed amount of chemicals in workers can only be assessed by the monitoring of the concentration in blood or urine. However, the determination of percutaneously absorbed amount is difficult due to the metabolism of the compounds as well as unknown and different individual factors. Such factors are especially the skin status and the use and efficacy of protective measures.

The risk of chemicals resulting from inhalation may be estimated by comparing the concentrations in the air with occupational threshold limit values derived by, e.g. the US ACGIH (American Conference of Governmental Industrial Hygienists) (11), the German DFG (Deutsche Forschungsgemeinschaft) (12), the Scientific Committee on Occupational Exposure Limit Values (SCOEL) (13), the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG) (14), or the Dutch Expert Committee on Occupational Safety (DECOS) (15). However, for genotoxic AA the risk estimation is sophisticated, since for such compounds occupational threshold limit values can not be derived (12) as, theoretically, one molecule is capable of causing cancer. This overview focuses especially on data in human skin, since animal data may show significant differences in percutaneous absorption as well as dermal metabolism of AA (16) and azo dyes being metabolized to AA (17).

3. SKIN BARRIER AGAINST CHEMICALS

Skin barrier against percutaneous absorption of exogenous substances is located mainly in the only ~20 µm thick stratum corneum. The structure of stratum corneum is like a "brick-and-mortar" model (18), where the hydrophilic corneocytes (bricks) are embedded into a lipid bilayer matrix (mortar). The resorption of exogenous substances occurs firstly in the capillary blood vessels located in the papillary layer beneath the epidermis in a distance of ~200 µm from skin surface. There are two routes for chemicals across the stratum corneum, the transcellular through the corneocytes and the intercellular through the lipid matrix (19). The intercellular route seems to be quantitatively more relevant than the transcellular route. De Jager et al. (20) showed that after extraction of intercellular skin lipids the percutaneous penetration of p-aminobenzoic acid and its derivatives in diffusion cell experiments increased significantly.

4. METHODS FOR THE ASSESSMENT OF PERCUTANEOUS ABSORPTION

The most important methods for the investigation of percutaneous absorption of chemicals are presented in Table 1. Percutaneous absorption of AA in workers can roughly be estimated from the blood concentration or more reliably from urinary excretion and the level of haemoglobin (Hb) adducts. A prerequisite for the interpretation of such data is a detailed knowledge of the metabolism and the excretion kinetics of the compounds. Whereas the urinary concentrations of AA reflect the current exposure, the Hb adducts assess the cumulative exposure of about 4 months according to the lifetime of the erythrocytes. Due to the low vapor pressures of AA (Table 2), biological monitoring is the most reliable method for the assessment of dermal uptake in workers.

Drexler *et al.* (21) described the estimation of percutaneous absorption from urinary excretion of chemicals considering their air concentration in the workplace

$$RIE = \frac{Chemical\ concentration\ in\ urine\ (\mu g / l)}{Chemical\ concentration\ in\ the\ air\ (\mu g / m^3)}$$

This ratio (RIE, relative internal exposure) increases with an increase in percutaneous absorption of a compound, allowing a standardization of the internal exposure. It means that all workers in a group would be exposed to the same air concentration of a chemical. RIE can compare the relationship between internal and external exposure of AA between individuals and estimate the part of percutaneous absorption contributing to the overall uptake.

Jakasa *et al.* (2) showed in volunteer studies using various concentrations of 2-butoxyethanol that the percutaneous absorption can be quantified from blood and urinary concentrations by comparison with inhalation data. Similar methodology was applied by the working group of Bader *et al.* (3). However, this method cannot be applied in humans for carcinogenic AA, where animal studies can be performed for the determination of fluxes with similar technique (22).

The most percutaneous absorption data of chemicals is obtained in diffusion cell experiments. There exist guidelines (23) and detailed consensus protocols (24) for such studies. Human *in vivo* and diffusion cell studies cannot be performed applying the same experimental conditions, although comparison studies between both methods are possible (25-29). These studies show, however, in part controversial results, i.e. an over- or underestimation of dermal penetration.

Microdialysis is a comparably new method to investigate the percutaneous absorption of chemicals showing some important advantages as well as drawbacks (Table 1) (30, 31).

5. PERCUTANEOUS ABSORPTION OF AROMATIC AMINES

5.1. Quantification of percutaneous absorption

The percutaneous absorption data for AA are often presented as absorbed percentage of exposed dose (16, 32). Such data are, however, less useful for practical approaches in occupational medicine, since for lipophilic compounds in most cases the higher the applied dose of a chemical, the lower the absorbed percentage. Therefore, an extrapolation of the data from this parameter is difficult

Table 1. Methods for the assessment of percutaneous absorption of aromatic amines

Method	Advantages	Problems	References
Biological monitoring			
In workers	▪ Real exposure situations	▪ Quantification of percutaneous absorption of chemicals is difficult	(21, 58, 69)
In human volunteers	▪ Real exposure situations can be closely imitated	▪ The methodological expenditure is high	(2, 3)
In animals	▪ Studies on a living organism	▪ Transferability of the data to human beings is questionable	(22)
Diffusion cell studies	▪ Human skin can be used ▪ Toxic and carcinogenic chemicals can be investigated	▪ Skin blood circulation is not imitated	(24, 40)
Microdialysis	▪ The technique is applicable in humans, animals or *ex vivo* skin ▪ Applicable in workers at workplace ▪ Pharmacokinetics can be studied ▪ Dermal metabolism can be investigated	▪ The methodological expenditure is high ▪ The technique must be calibrated ▪ Flux determination is difficult ▪ The percutaneous penetration of slowly permeable compounds can be significantly underestimated	(30, 31)

without a significant deviation from the real percutaneous absorption situation. For example, considering percutaneous penetration data from our laboratory (5) highly diluted aniline (*c*~0.003%) penetrated from an aqueous vehicle into the receptor fluid of diffusion cells to ~38% of applied dose. Applying an infinite dose of neat aniline only ~0.5% of the compound penetrated into the receptor fluid. However, considering the total penetrated amounts instead of the calculated percentage of absorption there were significant differences between the highly diluted (*c*~0.003%) and undiluted aniline (~6 vs. 2300 µg/cm^2 skin surface/24 h, respectively). The results suggest that the use of the parameter "absorbed percentage from applied dose" leads often to a significant underestimation of percutaneous absorption of a compound.

Another problem to quantify the percutaneous absorption of a chemical is when the data are provided as permeability coefficients (Kp). Kp values are still often presented in literature, since this parameter is considered to be independent from the concentration (33) or normalizable for any concentration of chemicals (34). As a consequence of this assumption the Kp is regarded to be constant for each chemical for the same vehicle. Considering a wide range of aqueous 2-butoxyethanol solutions, we showed that a prediction of fluxes from Kp values in literature is not possible for each concentration of the compound (35).

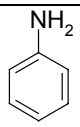
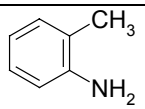
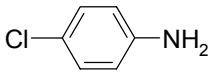
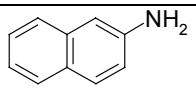
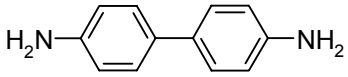
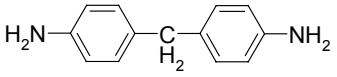
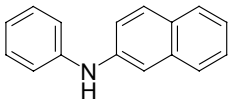
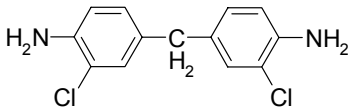
To quantify the percutaneous penetration of chemicals for the decision on skin notation the preferably used parameter is the flux, e.g. evaluated by the Ad hoc working group "percutaneous absorption" of the German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (4). The flux is defined as an amount of a chemical penetrated through a defined skin area and amount of time (unit: µg cm^{-2} h^{-1}). This parameter allows estimating quantitatively the percutaneous penetration of chemicals, when the exposed skin surface area (both hands: ~1000 cm^2; hands and forearms: ~2000 cm^2) and exposure duration are known. However, also the flux allows only an approximation of percutaneous absorption, since reliable fluxes can only be determined for certain exposure situations and the transferability of the data to other exposure conditions shows uncertainties.

5.2. Influence of physicochemical properties of aromatic amines on percutaneous absorption

Physicochemical properties of chemicals are often considered for a rough estimation of percutaneous absorption. Molecular weights and especially octanol-water partition coefficients (log P) of chemicals are accepted as main determinants of percutaneous penetration (36, 37). Considering the chemical structure and physicochemical properties, AA is a very heterogeneous group (Table 2). There are small (e.g. aniline, o-toluidine) and large (e.g. N-phenyl-2-naphthylamine (PBNA), 4,4'-methylene-bis(2-chloroaniline) (MOCA)) molecules, liquid (aniline, o-toluidine) as well as solid (e.g. benzidine, 2-naphthylamine) compounds. The smaller a molecule, the easier it usually penetrates through the skin. However, a molecular weight threshold for an optimum penetration of AA has not been evaluated. The log P values of AA are mostly higher than 0.8, indicating that a compound in an aqueous vehicle will penetrate to a lipophilic compartment, such as the lipid matrix of stratum corneum, instead to remain in the vehicle. An optimal range of log P determining the percutaneous absorption of AA is not evaluated. Stratum corneum contains a high percentage of lipid molecules, such as ceramides, free fatty acids and cholesterol (38). This could be the main reason that the most lipophilic AAs penetrate well through the skin, despite significant differences in chemical structure and a wide range in physicochemical properties (5). Furthermore, liquid AA tend to show higher fluxes than solid compounds (Tables 2-3).

AA are lipophilic compounds with a wide range in water solubilities (Table 2). The importance of water solubility of AA for the rate being percutaneously absorbed is less investigated. However, for aniline and o-toluidine the highest percutaneous penetration in terms of the percentage of applied dose was determined when the compounds were applied in aqueous solutions in the range of water solubilities (5). For o-toluidine, the difference was at a factor of ~100 compared to the undiluted application; for aniline, at a factor of ~10, when adjusting linearly for

Table 2. Physicochemical properties of some aromatic amines

Compound (CAS no.)	Chemical structure	Molecular weight (g/mol)	Water solubility (g/l)	Log P	Boiling point (°C)	Melting point (°C) (physical form [1])
Aniline (62-53-3)		93.13	36	0.90	184	–6 (liquid)
o-Toluidine (95-53-4)		107.16	16	1.32	200	–16 (liquid)
p-Chloroaniline (106-47-8)		127.57	3.9	1.83	232	73 (solid)
2-Naphthylamine (91-59-8)		143.19	0.189	2.28	300	113 (solid)
Benzidine (92-87-5)		184.24	0.322	1.34	401	120 (solid)
4,4'-Methylenedianiline (MDA) (101-77-9)		198.27	1	1.59	398	93 (solid)
N-Phenyl-2-naphthylamine (PBNA) (135-88-6)		219.29	0.006	4.38	396	108 (solid)
4,4'-Methylene-bis(2-chloroaniline) (MOCA) (101-14-4)		267.16	0.014	3.91	379	110 (solid)

[1] At room temperature

the applied concentration. Moreover, considering the percutaneous penetration of aniline there is a linear relationship between the dermal fluxes and the concentration of the compound in aqueous vehicle nearly to saturation (5). A similar relationship applies also to o-toluidine.

5.3. Influence of dose and vehicle on percutaneous absorption

Percutaneous absorption data for AA have been obtained using different doses and various vehicles, such as aqueous solutions (5), acetone (32), ethanol (39), workplace specific lubricant (40) or other. This makes a direct comparison of the data difficult. There is no evidence that experimentally determined fluxes, obtained using a certain vehicle, can be transferred without restrictions to other vehicles. It means that percutaneous absorption data have to be specifically generated for various occupationally relevant concentrations and exposure situations. Our experimental data for aniline in aqueous vehicle (5) indicate that a linear extrapolation of the flux up to the water solubility limit of the compound is possible. The same relationship was also demonstrated for the structurally similar solvents toluene and styrene in humans (41).

In our study applying o-toluidine in a silicone-free polymer lubricant, a slight percutaneous penetration enhancement of the AA was found (40). Baynes *et al.* (32) report in a diffusion cell study using pig skin that there is a penetration enhancement of benzidine applied in chemical mixtures containing solvents (acetone, dimethyl sulfoxide) and surfactants (methyl nicotinate, sodium lauryl sulfate). However, the data of the latter study are difficult to interpret, since no control experiments were conducted. Incorporation of nanoparticles into the vehicle led to an increase of the percutaneous penetration of chemicals. Küchler *et al.* (42, 43) reported that the azo dyes "Rhodamin B" and "Nile Red" penetrated in diffusion cell experiments faster through the skin from nanoparticle cream than from conventional cream formulations.

5.4. Dermal metabolism of aromatic amines

The general principle of the dermal metabolism of AA should mainly base on the cytochrome P-450 (abbr. CYP) enzyme family. An important phase I reaction of AA is the oxidation. It will be triggered by the enzyme CYP1A2, which is also expressed in human keratinocytes (44, 45). Other enzymes of the CYP1A family were also detected in the skin (46) indicating enzyme overlapping in the activation of AA. It is assumed that the phenolic metabolites represent quantitatively the main group, which will be further acetylated and conjugated in the phase II reactions. Collier *et al.* (17) assumed that aniline was metabolized in excised human skin probably by acetylation.

Table 3. Toxicological relevance of percutaneous penetration for some aromatic amines

Compound	Skin notation[1]	Flux [2] (μg cm^{-2} h^{-1})	TLV-TWA level (mg/m^3) [3]	Skin exposure time up to TLV-TWA level (min) [4]
Aniline	H; Skin	218 (*c*~3%)	7.7 (2 ppm)	21
o-Toluidine	H; Skin	117 (*c*~100%)	8.9 (2 ppm)	46
4,4'-Methylenedianiline	H; Skin	34 (*c*~0.5%)	0.8 (0.1 ppm)	14
4,4'-Methylene-bis(2-chloroaniline) (MOCA)	H; Skin	~4 times lower than MDA flux	0.1 (0.01 ppm)	7

[1] German DFG (12) and US ACGIH (11), [2] Fluxes and concentrations (*c*) were obtained or estimated from the data of Wellner *et al.* (5) and Hotchkiss *et al.* (16), [3] TLV-TWA: occupational air threshold limit value (mg/m^3) - time weighted average exposure on the basis of a 8h/day, 40h/week work schedule (11), [4] Values were calculated for 1000 cm^2 skin surface (both hands). The inhalative uptake of aromatic amines at the TLV-TWA level was calculated for 10 m^3 air, which corresponds to the inhalation volume for 8 hours.

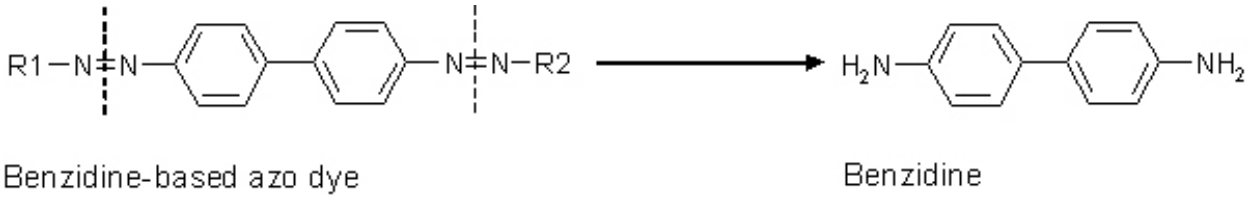


Figure 1. Reduction of a benzidine-based azo dye to benzidine.

However, there is a lack of data on the activity and the capacity of such enzymes in the skin compared to the liver.

Recently, a significant capacity of dermal metabolism was shown for the monocyclic AA p-aminophenol in reconstructed epidermis (47). p-Aminophenol was completely metabolized within 24 hours, indicating that N-acetyltransferase (NAT1) mediated metabolism was a major route of AA detoxification following dermal exposure. This metabolic pathway for p-aminophenol was also described using human keratinocytes (48). The same detoxification route (NAT1) was also assumed for p-phenylenediamine in a volunteer study after dermal exposure (48). The authors concluded that the key enzyme in the detoxification of topically applied AA appeared to be the skin NAT1 rather than hepatic NAT2.

The enzyme activity in freshly excised skin can be maintained in diffusion cell experiments. A high rate of conjugation was observed for dinitrochlorobenzene (DNCB), an aromatic nitro compound (49). Whereas in low dose exposure (25 μl/cm^2 skin) the conjugated DNCB penetrated into receptor fluid ~2-fold faster than the unchanged DNCB; in experiments applying a 5-fold higher DNCB dose this ratio was lower (~1:4), but still significant. These findings indicate, however, a limited extent of glutathione-S-transferases in excised skin catalyzing this reaction. Aromatic amino and nitro compounds are structurally similar and both often present at workplaces. For other lipophilic compounds with similar physicochemical properties, such as the polycyclic aromatic hydrocarbon benzo[a]pyrene, the dermal metabolism may reach a high double-digit range (50). Therefore, the dermal metabolism of AA should be taken into account as a possible modifier of percutaneous absorption to avoid a significant underestimation of the uptake.

5.5. Percutaneous absorption and dermal metabolism of azo dyes

Azo dyes are characterized by the presence of one or more azo groups (−N=N−). The interest of experts for occupational medicine and hygiene arises from epidemiological observations that occupational exposure to azo dyes can increase the incidence of bladder cancer (12, 51). Percutaneously absorbed azo dyes can be metabolized by intestinal bacteria or by azo reductases of the liver and extrahepatic tissue to corresponding AA, and in this way increase the internal exposure to AA. Figure 1 shows the principle of the reduction of azo dyes to benzidine as an example.

It is assumed that enzymes being expressed in the liver are also available in the skin and a dermal metabolism of azo dyes is expected. Therefore, all azo dyes, from which carcinogenic AA can be cleaved, are considered to have carcinogenic potential (12). Of particular toxicological interest are azo dyes from doubly diazotized benzidine and some benzidine derivatives. But also other carcinogenic AA, such as 2-naphthylamine, 4-aminodiphenyl, 4-chloro-o-toluidine, and o-toluidine can be metabolized from the corresponding azo dyes. There are only a few data available on percutaneous absorption of azo dyes, which could be metabolized to carcinogenic AA. Aldrich *et al.* (52) showed in animal experiments (rabbits and rats) the capability of benzidine-based azo dye "Direct Black 38" to penetrate through the skin. Collier *et al.* (17) found in diffusion cell studies with human skin that all 3 tested azo dyes ("Yellow No. 6", "Sudan I", and "Solvent Yellow 7") penetrated within 24 hours at ~5–40% of the applied dose.

AA may also be formed from azo dyes on skin surface by bacterial flora. Table 4 presents data on metabolism of azo dyes to AA by human skin enzymes and by skin surface bacteria. For example, bacterial reduction of the azo dye "Direct Blue 14" led to the formation of o-

Table 4. Metabolism of azo dyes by human skin enzymes or skin surface bacteria to aromatic amines

Azo dye	Metabolite	Causes for metabolism	% of dye metabolized	Reference
Sudan I	Aniline	Skin enzymes	29.5 ± 0.5	(17)
Solvent Yellow 7	Aniline	Skin enzymes	26.5 ± 1.9	(17)
Direct Blue 14	O-Toluidine	Skin surface bacteria	N.d. [1]	(53)
Disperse yellow 3	4-Aminoacetanilide 2-Amino-p-cresol	Skin surface bacteria	N.d. [1]	(54)

[1] N.d.: not determined

toluidine (53). A rise of the number of bacteria in the assay increased proportionately the cleavage of "Direct Blue 14". Most of the bacterial strains of normal human skin flora exhibit reductive activity and cleave "Direct Blue 14" in a synthetic sweat suspension (53). Many other AA, such as 4-aminoacetanilide, 2-amino-p-cresol (54), p-nitroaniline, 2,6-dichloro-4-nitroaniline and p-phenylenediamine (55), may exhibit cleavage-products of azo dyes.

The reductive cleavage potential of several skin surface bacteria may achieve 100% within less than 5 h of exposure as demonstrated on the azo dyes "Methyl Red" and "Orange II" (56). The higher molecular weight of azo dyes, the slower percutaneous absorption of the compounds could be expected. Additionally, it was demonstrated that sweat would enhance the percutaneous penetration of chemicals (57). Therefore, the presence of sweat on skin surface, e.g. by wearing of textiles and leather treated with azo dyes, could optimize the conditions for cleavage of azo dyes and increase the percutaneous absorption of formed AA.

5.6. Influence of personal protective equipment on percutaneous absorption

From the point of view of percutaneous absorption, gloves and skin creams are the most important personal protective measures influencing the internal exposure to AA. The wearing of personal protective equipment can, however, either reduce the percutaneous absorption of AA or increase it, depending on the efficacy of the measures.

Recently, we reported that skin barrier creams did not prevent workers in the German rubber industry from a relevant systemic uptake of aniline and o-toluidine, comparing the internal exposure with that in the general population (58). Considering the relative internal exposure (RIE; see chapter 4) in a multiple linear regression analysis it was evident that the use of skin barrier creams plays an important role of increasing the uptake of these AA. Subsequent diffusion cell experiments confirmed that the application of skin barrier creams enhanced the percutaneous penetration of aniline and o-toluidine significantly compared to untreated human skin (40). The enhancement was independent from the formulation (oil-in-water or water-in-oil emulsion) of skin creams.

On the other hand, the frequent wearing of gloves reduced significantly the internal exposure to aniline and o-toluidine in workers in the rubber industry compared to workers, who used gloves less frequently (58). In a study applying a modified ASTM F739 method, which is considered to be a standard method for testing the permeation of liquid chemicals through protective clothing materials under conditions of continuous contact, the breakthrough times of aniline among 5 glove materials ranged from only ~3 to 82 min (59). Only nitrile butyl rubber gloves showed satisfactory protection for 51–82 min, whereas the breakthrough time for polyvinyl chloride (PVC), natural latex rubber, polymerized alkene, and nitrile material ranged from only 3 min for nitrile to ~12 min for PVC. The nitrile butyl rubber glove material was, however, at least 2-times thicker than the other materials. Kenyon *et al.* (10) observed in diffusion cell experiments using latex or nitrile glove materials that up to 5% of 4,4'-methylenedianiline (MDA) (applied dose: 0.1 mg) penetrated from an ethanol-water (50:50) vehicle within 5 h of exposure, but for nitrile gloves the penetration of MDA was much lower (0.1%). All these factors, and especially the lack of data, make the evaluation of the efficacy of personal protective measures to prevent the dermal uptake of AA and azo dyes difficult.

6. RISK ASSESSMENT FROM PERCUTANEOUS ABSORPTION

Evidence-based risk assessment is difficult for each chemical with toxic potential when adequate data are insufficient. For AA showing heterogeneous physicochemical properties, the performance of studies with the same concentrations and vehicles is difficult. The low vapor pressures and high boiling points of AA (Table 2) lead to low, often not detectable concentrations in the workplace air.

Human studies are considered to be the "gold standard" to generate percutaneous absorption data (19, 23). For carcinogenic AA, percutaneous absorption data can only be derived from animal and *ex vivo* studies. However, there may be large over- as well as underestimations of percutaneously absorbed amounts between the species, where the difference may reach even a factor of 100 (60). Whether animal data can be transferred without any restrictions to human beings has been less investigated. Therefore, in risk assessment studies evaluating the contribution of percutaneous absorption of toxic chemicals to overall uptake studies in human skin are preferred (23, 61). Data from diffusion cell experiments using excised human skin match closely to data obtained in humans (29) and are increasingly accepted for practical and regulatory purposes (61).

The most valuable and preferred parameter for the calculation of percutaneous absorption in risk assessment purposes of occupational exposure is the maximum flux, which could reduce significant

underestimations of dermal absorption. Cumulative working life absorption of AA already in a low milligram range is considered to be capable of causing bladder cancer (62). To estimate the risk the percutaneously absorbed amount of AA should, therefore, be compared with the occupational threshold levels. When a linear dose-absorption relationship, as showed for aqueous aniline solutions (5), would also exist for other AA, this would simplify a semi-quantitative risk assessment. Kenyon *et al.* (10) described for MDA a linear dose-absorption relationship in a 50% (v/v) aqueous ethanol vehicle. It means that percutaneous penetration data can be extrapolated linearly to other concentrations. However, there are not enough data for AA for a general appliance of this observation.

The health risk resulting from occupational skin contact to chemicals can be assessed considering the skin notations ("H" in Germany, "skin" in USA), which are assigned when the percutaneous absorption significantly contributes to the overall uptake (63). However, there is no uniform skin notations assignment for chemicals in industrial countries (64). For example, in Germany 2-naphthylamine is assigned with "H" (12) since many years, whereas in the TLV and BEI list of ACGIH "skin" is still missing (11), although the carcinogenic potential and the percutaneous penetration of the compound is one of the highest among all AA (5).

In cases of insufficient data, the appliance of a reasonable worst-case exposure scenario is recommended for risk assessment (65). Considering the example of pesticides, 100% default absorption has been adopted as a conservative approach for the risk assessment in case of the absence of data (66). Similarly, for large compounds with high log P values, a default percutaneous absorption of 10% of the exposed dose could be discussed. In principle, default values between 10% and 100% can be made on a case-by-case expert judgment. When molecular weights, water solubilities and log P of AA are known, mathematical models based on these properties could be applied to predict the percutaneous penetration (12). However, the predictive potential of such models is weak (4). For example, for aniline, the difference between the experimentally determined and the by the model of Guy and Potts (67) predicted flux was at a factor of ~9. When mathematical models cannot be applied, e.g. due to insufficient data on physicochemical properties, an estimation of percutaneous absorption could be made on the basis of data for structurally analogous compounds (12). However, this approach can only be used for a rough estimation of percutaneous absorption of chemicals.

A ranking of percutaneous penetration among AA could also be useful for the risk assessment. Such an approach is possible for some AA considering the data for MDA in the studies of Wellner *et al.* (5) and Hotchkiss *et al.* (16). From the data of these studies an approximate percutaneous penetration ranking of diluted AA can be assumed: 2-naphthylamine > o-toluidine > aniline > MDA > MOCA > PBNA. Levillain *et al.* (68) determined in a diffusion cell study in rat skin a percutaneous penetration ranking for other AA: p-chloroaniline > m-trifluoromethylaniline > dichloro-3,4-aniline > dichloro-3,5-aniline. Using the mathematical model of Guy and Potts (67), the flux ranking for these compounds is: p-chloroaniline > MDA > benzidine. Due to significant differences in experimental protocols, a ranking for all these AA is, however, difficult. Considering all these data the percutaneous penetration ranking of the 11 AA could be: 2-naphthylamine > o-toluidine > aniline > p-chloroaniline > MDA > MOCA > m-trifluoromethylaniline > dichloro-3,4-aniline > dichloro-3,5-aniline > benzidine > PBNA, where experimentally determined fluxes were weighted higher than mathematically predicted fluxes.

For the risk evaluation of the percutaneous absorption of AA in workers, the dermal uptake can be compared, e.g. with NOAEL (no observed adverse effect level), LOAEL (lowest observed adverse effect level), ADI (acceptable daily intake), TDI (tolerable daily intake) or IDLH (immediately dangerous to life and health) values. Already short-term dermal exposure to AA is of toxicological relevance. For example, for aniline, o-toluidine, MDA or MOCA the dermal absorption may exceed the uptake by inhalation often within less than one hour of exposure (Table 3). 2-Naphthylamine and o-toluidine show genotoxic potentials and, therefore, no dose-effect relationships can be derived for these AA. In a previous study (5), we compared the dermal uptake of aniline with the IDLH value (387 mg/m^3). A continuous contact of the hands to a 3% aqueous aniline solution (flux: 218 µg cm^{-2} h^{-1}) over 8 h could reach the uptake by inhalation (10 l air/min) at the IDLH value (~1744 vs. 1858 mg). In workers exposed to AA, biological monitoring is the method of choice for assessing the risk of dermal absorption (63).

In Germany, the Technical Rules for Hazardous Substances (TRGS) reflect the state of requirements in terms of safety, occupational health, hygiene, and work science relating to activities involving hazardous substances. The TRGS no. 614 deals with azo dyes. The dermal exposure should be estimated by the quantification of the amounts of exposed azo dyes and on skin surface formed AA. From the preventive point of view it is recommended that azo dyes should be dealt with as if they were classified in the same carcinogenic category as the corresponding AA (12).

7. SUMMARY AND PERSPECTIVE

The percutaneous absorption of AA contributes significantly to the overall internal exposure in workers. The dermal uptake of AA may exceed the uptake by inhalation often within less than one hour of exposure. We assume that AA being absorbed percutaneously are capable to cause systemic toxic effects in the same manner as by inhalative uptake.

For carcinogenic AA, an appliance of a reasonable worst-case exposure scenario can be recommended for workplaces to avoid a significant underestimation of internal exposure resulting from dermal

absorption. The maximum flux seems to be the most valuable parameter for the risk assessment. For this purpose, the percutaneously absorbed amount of AA can be compared with the maximum uptake at occupational threshold limit values in the air, e.g. the US TLV-TWA (threshold limit value - time weighted average) or the German MAK values (maximum workplace concentration), which have been derived considering toxicological aspects. From the toxicological point of view the dermal exposure of azo dyes, which can be metabolized to carcinogenic AA, should be considered similarly to the exposure of those AA.

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Abbreviations: AA: aromatic amines; MDA: 4,4'-Methylenedianiline; MOCA: 4,4'-Methylene-bis(2-chloroaniline); PBNA: N-Phenyl-2-naphthylamine; TLV-TWA: occupational air threshold limit value - time weighted average

Key Words Aromatic amines; Azo dyes; Percutaneous absorption; Dermal metabolism; Biological monitoring; Risk assessment, Review

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